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Review

Artificial and Bioartificial Liver Support

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ABSTRACT

The fact that liver failure constitutes a life-threatening condition and can, in most cases, only be overcome by orthotopic liver transplantation, lead to the development of various artificial and bioartificial liver support devices. While artificial systems are based on the principles of adsorption and filtration, the more complex concept of bioartificial devices includes the provision of liver cells. Instead of solely focussing on detoxification, these concepts also support the failing organ concerning synthetic and regulative functions.

The systems were evaluated in a variety of clinical studies, demonstrating their safety and investigating the impact on the patient's clinical condition. This review gives an overview over the most common artificial and bioartificial liver support devices and summarizes the results of the clinical studies.

INTRODUCTION

The liver is a complex organ with various vital functions in synthesis, detoxification and regulation; its failure therefore constitutes a life threatening condition.[1] Liver failure (LF) can either occur without preceding liver disease (acute liver failure, ALF), usually caused either by intoxication (Amanita phalloides, acetaminophen, methylendioxymethamphteamine) or as acute decompensation of chronic liver-related illness (acute-on-chronic liver failure, AoCLF). In both cases, its symptoms include icterus, hepatic encephalopathy and impairment of coagulation status and may result in multi organ failure. Exceptionally, liver failure may also be triggered by certain diseases (Budd-Chiari-syndrome, Morbus Wilson) or pregnancy.

The only long-term therapy in most cases is orthotopic liver transplantation, unless the liver is able to regenerate. Many patients, especially those who are not listed for high urgency transplantation, may not survive until a suitable donor organ is available, since donor organs are rare. In other cases, contraindications do not permit liver transplantation. For these indications, extracorporeal liver assist devices have been developed in order to either bridge the patient to transplantation or temporarily support the failing organ until it is able to regenerate.

In the course of liver failure, water-soluble toxins (e.g., ammonia, mercaptans) and albumin-bound toxins (e.g., bilirubin, bile acids, aromatic amino acids, fatty acids) may accumulate and cause encephalopathy and dysfunction of other organs. While the field of detoxification and partially also of regulation can be addressed by artificial devices similar to dialysis (artificial systems, detoxification devices), the synthetic function of the liver can only be provided by living cells. In order to temporarily apply these cells in a safe and convenient way, bioartificial liver support devices were developed.

ARTIFICIAL SYSTEMS

Cell free artificial systems make use of the processes of adsorption and filtration, assuming that removal of toxins from the patient's plasma will improve the clinical state of the patient. In the early developmental stages, the devices were solely able to remove a certain portion of water-soluble toxins.

Haemodialysis, being the common treatment for renal failure, is also used for treatment of patients in liver failure to remove water soluble toxins. Since liver failure is often accompanied by renal failure, haemodialysis is part of the standard intensive care treatment.

In charcoal haemoperfusion, patient plasma is separated and led trough activated charcoal filters, which are able to adsorb a variety of water-soluble toxins in the

low and middle molecular weight range, these being involved in the symptoms of LF. Initially, this process presented difficulties in terms of biocompatibility, loss of thrombocytes and clotting problems, which could partially be overcome by avoiding direct contact of plasma and charcoal particles. Numerous clinical studies were carried out in the 70's and 80's[2,3] but no significant survival benefit could be stated for this method.[4]

For plasma exchange/plasmapheresis, the cellular components of the blood are separated from the plasma using a plasma filter. Plasma is then replaced by either fresh frozen plasma, albumin solution or other plasma substitutes. By this method, certain toxins present in the plasma are removed.[5,6] However, this method requires a large plasma stock and bears the risk of infections.

All previously mentioned methods, although resulting in improved biochemical and clinical conditions and removal of toxins, did not substantiate a survival benefit for the patients. To control the versatile toxins, more sophisticated detoxification systems were developed, also taking into account the elimination of lipophilic, albumin bound toxins. The two main resulting concepts are albumin dialysis and fractionated plasma separation.

The Molecular Adsorbent Recirculation System (MARS) banks on the recycling of albumin solution via an anion exchanger and active charcoal. The patient's blood is led through the hollow fibre capillaries of a high flux dialysis filter. Albumin solution, which is circulated in the extracorporeal circuit, passes the membrane counter directionally, allowing albumin-bound toxins in the blood to cross the membrane and bind to the albumin of the MARS circuit. The membrane is, however, impermeable to albumin. When passing the adsorber and filter cartridges, the toxins are cleared by the filter and albumin is regenerated and able to accept new toxins when passing the membrane again. Additionally, the albumin circuit itself is dialysed in the method of continuous veno-venous haemodialysis (CVVHD) or continuous veno-venous heamodiafiltration (CVVHDF), diminishing the load of water-soluble toxins.

According to Li,[7] a similar but less expensive system (continuous albumin purification system (CAPS)), using commercially available dialysis cartridges and adsobers, had been developed in China.

MARS is frequently applied to patients with liver failure and several single-center experiences and nonrandomized trials have been published.[8-11] In a first randomized controlled trial,[12] thirteen patients with cirrhosis were divided into two groups: A control group (n = 5) receiving standard medical treatment and hemodiafiltration (HDF), and a group (n = 8) additionally being treated with MARS. The MARS-treatment was applied 1–10 times for 6–8 hours. A significant decrease in creatinine and bilirubin levels as well as an increase in serum sodium level and prothrombin activity was detected in the MARS group. Mortality of the control group reached 100% after seven days, whereas it was at 62,5% in the MARS group. The 30-day-survival lay at 75%, presenting a significant prolongation of survival in the long-rank test.

The MARS clinical data has been recently summarized by Chiu and Fan,[13] concluding that, although there is no substantial data demonstrating an overall survival benefit with MARS treatment, there are certain subgroups of liver failure, such as ALF and graft dysfunction, showing promising results with MARS.

Single Pass Albumin Dialysis (SPAD) applies similar principles. The patient's blood also passes a high flux dialysis membrane. Albumin solution streams along the other side of the membrane counter-directionally, accepting toxins from the plasma. However, in SPAD the albumin solution is discarded after a single passage of the membrane without being recycled. The concept enables CVVHDF using the same dialysis filter.[14]

MARS, SPAD and CVVHDF were compared in an in vitro-study concerning the efficiency of removing both albumin-bound and water-soluble toxins.[15] No significant difference was found in the removal of water-soluble toxins for all three systems. Albumin bound toxins were removed to a similar extent by MARS and SPAD, both showing a significantly higher efficiency than CVVHDF.

The Prometheus system is based on fractional plasma separation and adsorption (FPSA) and haemodialysis. It uses a membrane with a cut-off of 250 kDa, being permeable for albumin. The toxin-loaded patient albumin crosses the membrane and passes a neutral resin adsorber and an anion exchanger, where the toxins bind to the adsorbers and free albumin is brought back to the patient. The method is combined with additional haemodialysis, therefore being able to remove water-soluble toxins as well as albumin-bound toxins.

A small clinical study was performed including nine patients with AoCLF and documented cirrhosis due to alcohol or chronic viral infection, to confirm the efficacy of the system, to outline the effect of the single components and to evaluate the saturation effect of the adsorber columns.[16] It was shown that water-soluble toxins were almost exclusively cleared by the dialyzer, whereas bilirubin was cleared by the adsorber column, as expected. However, the clearance of bilirubin and bile acids strongly decreased in time, suggesting a saturation of the adsobers. In general, the Prometheus system was shown to be effective in the removal of various toxins and to trigger no adverse events.[17-20]

In a comparative study, nine patients were treated with MARS and Prometheus, respectively.[21] Treatment was performed for at least 5 hours on 2–5 consecutive days, using the same blood and dialysate flow rates for both systems. Prometheus showed significantly higher reduction rates for all markers except bile acids for the overall treatment as well as for single treatment. It was shown that the clearance of protein-bound substances declined over time in MARS but not in Prometheus.

In Selective Plasma Filtration Therapy (SEPET) the patient's blood is lead through a single-use cartridge containing hollow fibres with a molecular weight cut-off at 100 kDa. A plasma fraction containing several of the accumulated toxins in the blood is discarded after passing the membrane. This fraction contains toxins of small molecular weight and free pro-inflammatory cytokines but not for example immunoglobulins. Molecules with a molecular weight close to 100 kDa pass the membrane in only limited amounts so that large portions of for example albumin, HGF etc, as well as several clotting factors, are retained. The fluid loss is replaced by electrolyte solution, human albumin solution, fresh frozen plasma or a combination thereof. The system is designed for use with any commercially available kidney dialysis unit and/or plasma apheresis system utilizing hollow-fibre cartridges.

An animal study in a fulminant hepatic failure (FHF) pig model was performed,[22] showing a significantly longer survival time for pigs treated with SEPET in comparison to sham-SEPET (immediately returning the separated plasma fraction). No side effects were shown and the system effectively removed the investigated toxins. A phase-I clinical study with AoCL patients is currently being performed.

BIOARTIFICIAL SYSTEMS

Bioartificial systems were developed to take over partially the synthetic and regulatory function of the liver besides detoxifying the patient's plasma. They use liver cells as biological component to accomplish this task. Different cell sources have been utilized in several systems. In general, there are the following options: using primary cells, either human or of xenogeneic derivation, cell lines (tumor cell lines or immortalized cell lines) or developing expandable progenitor cell populations.

Primary human cells meet the demand of biocompatibility. Those cells can be isolated from donor organs rejected for transplantation, however, the logistics of receiving human organs and isolating cells are too complicated to provide systems for large clinical studies. Xenogeneic cells, usually of porcine origin, are more easily available; however, there is a certain risk of infections and the metabolic compatibility is not assured. Most currently available liver cell lines display only a fraction of the metabolic activity of primary human liver cells, so that presumably a very large cell mass has to be applied to display any therapeutic success.[23,24] Secondly, although the cells are separated from the patient's blood stream by capillary membranes and additional filters, the risk of metastasis formation is not to be excluded.

Considering those aspects, the ideal cell source would probably be human progenitor cells, however, until today it is impossible to expand and differentiate liver progenitor cells in culture to a sufficient cell mass.

The *CellModule* is part of the Modular Extracorporeal Liver Support (MELS) device and can be combined with continuous veno-venous haemodiafiltration and albumin dialsysis.[25] The *CellModule* bio-reactor consists of three interwoven hollow fibre bundles which are embedded in a polyurethane housing.[26] Two bundles of hydrophilic polyethersulfone membranes with a pore size of 0.5 ± 0.1 μm serve for culture medium or plasma perfusion while one bundle of hydrophobic multilaminate fibres is used for decentralized oxygenation. By mimicking the vessel structure of the liver, those hollow fibres form small repetitive units and assure the supply of the cells with oxygen and nutrients. The system can be operated with up to 600 g of liver cells, which are inoculated in the inter-capillary space via 24 open-ended silicone tubes. During therapy, the patient's plasma is separated from the blood cells via plasma filter and recirculated through the hollow fibres at 200–250 ml/min.

Initially, the system was inoculated with primary porcine liver cells obtained from specific pathogen-free (SPF) pigs. In a clinical phase-I-study,[27] eight patients with ALF were treated for 8–46 hours. All patients were listed for high urgency transplantation. No complications occurred during therapy, all patients were successfully bridged to transplantation and the follow-up showed a five-year survival of 100%. No infection with porcine endogenous retroviruses (PERV) could be detected in any of the patients.[28]

With the rising discussion about xenogeneic infections and the question of whether or not porcine cells are completely compatible with the human liver metabolism, primary human liver cells isolated from discarded donor organs were explored as an alternative cell source. In the context of a phase-I-study, twelve patients with ALF, AoCLF or primary graft non-function (PNF) were treated with MELS for 10–270 hours. Patients with ALF and PNF were successfully bridged to transplantation, whereas three out of six patients with AoCLF, who were contraindicated for transplantation, died within three month after therapy. Overall, an improvement of neurologic status, kidney function and toxin levels could be observed under therapy and the system proved to be biocompatible and safe.[29] A case report of a patient with PNF who was treated with the complete MELS system including CVVHDF and SPAD documents explicit improvement of the clinical status under therapy.[30]

In contrast to all other systems discussed in this review, the bioartificial liver of the Academisch Medisch Centrum Amsterdam (AMC-BAL) does not separate the cells from the patient's plasma by capillary membranes. A spirally-wound mat of nonwoven polyester fibers inside a housing provides attachment area for the liver cells. Oxygenation capillaries are incorporated by the matrix to provide local oxygenation. 10×10^9 primary porcine liver cells are seeded in the matrix, where they are able to adhere. During therapy, the patient's plasma is directly perfused through the matrix, so this system features only one membrane barrier, one fewer than most other bioartificial livers, and enables direct cell-plasma-contact.[31,32] In a clinical phase-I-study, twelve patients with ALF awaiting high urgency transplantation were treated with the AMC-BAL for 4–35 hours. Four of the patients received two treatments within three days. All patients showed improvement of neurological state and diuresis as well as stabilization of haemodynamics. Eleven patients were successfully bridged to transplantation, one patient showed improved liver function after two treatments and did not require transplantation.[33,34] Generally, the treatment showed no adverse events.

MELS and AMC-BAL were compared in an in vitro study with porcine cells.[35] This was so far the first attempt to directly compare two bioartificial liver support systems under similar conditions, showing that the cell performance was similar in both bioreactor types, only showing minor differences in some parameters.

The Extracorporeal Liver Assist Device (ELAD) utilizes C3A cells, a cell line derived from the human hepatoblastoma cell line HepG2. The cells are localized in the extracapillary space of a modified dialysis cartridge. The membrane cut off of 70 kDa was chosen to prevent immunoglobulins and blood cells as well as C3A-cells from crossing. Two additional cell filters assure that no tumour cells can reach the patient's blood stream.[36]

The early ELAD consisted of four cartridges, each filled with approximately 50 g of cells and blood was led through the cartridges at 150–200 ml/min. In a phase-I-study, eleven patients were treated, ten of whom showed an improvement in biochemical status. Four patients were successfully bridged to transplantation and one patient recovered without transplantation. The safety of the system was proven.[37]

A clinical pilot controlled study included 24 patients with ALF caused by intoxication or viral hepatitis.[38] Patients were divided in two groups, those with substantial chance of spontaneous recovery (17 patients, group I) and those already listed for high urgency transplantation (seven patients, group II). The groups were each divided in two subgroups: Nine patients in group I and three patients in group II received ELAD treatment, leaving a control group of eight and four patients receiving standard treatment, respectively. ELAD was performed for 3–72 h in group I and up to 168 h in group II. Two cartridges were used in the system, providing a cell mass of 400 g. Biocompatibility and safety of the device was shown, and a slightly higher rate of improvement in encephalopathy and an increase in galactose elimination capacity after 6 h of treatment could be seen in the ELAD-patients. However, no significant survival benefit could be demonstrated (78% and 75% in group I and 33% and 25% in group II respectively), partly being attributed to the fact

that the recovery rate in the group I control group was much higher than the predicted 30–50%.

In the modified ELAD, 100–200 g of cells were inoculated in each cartridge and the system was perfused with ultrafiltrate (membrane cut-off 120 kDa) instead of whole blood. The flow rate was increased to 500 ml/min. Five patients with ALF were included in a pilot study and treated for 12–107 h.[39] All patients were successfully bridged to transplantation and four out of five survived until the 30-day endpoint of the study.

The HepatAssist is operated with 5–7 x 10^9 porcine liver cells which are cryopreserved until clinical application. The cells are also located in the extracapillary space of a modified dialysis cartridge. The patient's plasma is lead through the bioreactor after passing an active charcoal filter and an oxygenator first.[40,41]

In a phase-I-study, seven patients with ALF were treated for 6–7 hours. The system proved to be safe and the patients' neurological status improvement under treatment.[42] In another uncontrolled trial, 39 patients divided in three subgroups [group 1: patients in ALF listed for transplantation (n = 26); group 2: patients with primary graft nonfunction (n = 3); group 3: patients with AoCLF (n = 10)] were treated.[43,44] In group 1, 18 patients were successfully bridged to transplantation, one of them dying on day 7 post transplantation. Six patients in this group, five of whom suffered from acetaminophen-induced LF, recovered without liver transplantation. Two patients died before transplantation could be performed. All patients in group 2 were successfully bridged to retransplantation. All patients in group 3 showed temporary improvement in their clinical state, however, only two survived the AoCLF while eight patients died within 21 days.

The so far largest prospective, randomized, controlled trial[45] included 171 patients with fulminant/subfulminant liver failure and PNF. 86 patients were assigned to the control group receiving standard medical treatment and 85 patients were additionally treated with HepatAssist. There was no significant improvement in 30 day-survival. When assessing the relative risk, there was no significant effect when regarding the complete study population, however, in the group of only fulminant/subfulminant hepatic failure (excluding PNF), there was a significant decrease in relative risk (relative risk = 0.56; p = 0.048). Time to death was not significantly prolonged in the HepatAssist group when looking at all patients, but excluding PNF and LF of unknown etiology (remaining n = 83), a significantly prolonged survival could be found in the HepatAssist group (p = 0.009).

There are several limiting aspects which generally have to be taken into account when talking about bioartificial liver support. In most bioartificial liver support devices, the liver cells are separated from the patient's blood or plasma by at least one membrane. This provides an immunological barrier, but also limits the exchange of substances and therefore potentially reduces the effectiveness of the system. Furthermore, the blood/plasma flow is limited to 100–300 mL/min, whereas the blood flow in a normal human liver is about 1500 mL/min, additionally diminishing the possible maximum clearance.[46]

It is known from partial hepatectomy and split liver transplantation experience, that a minimum liver mass is necessary to provide sufficient liver function for survival. In living donor split-liver transplantation, the risk for graft nonfunction is articulately higher when the graft mass is less than about 40% of the ideal liver mass of the patient.[47] The question remains whether cell masses of around 50–200 g used in most bioartificial liver devices will show a significant effect, even when disregarding the limitation by membrane barriers.

In a recent Crochane Review, trials of artificial and bioartificial liver support devices either compared to standard medical treatment (483 patients) or compared to other support systems (105 patients) were summarized. The authors found no general effect on survival in ALF, but a slight effect in AoCLF. They suggest further randomized multicenter studies with larger case numbers.[48] A similar conclusion is reached by another systematic review surveying in total 353 patients with ALF and 130 patients with AoCLF.[49]

In conclusion, the approach of bioartificial liver support in combination with artificial devices is promising but several challenging tasks, such as identifying the ideal cell source and the agreement on a more uniform study design, still remain. The goal is to develop a liver support device that will grant patients a substantial survival benefit compared to standard intensive care and to prove this in an appropriate randomized clinical study.

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